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Novel carbon–carbon bond formations for biocatalysis

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Carbon–carbon bond formation is the key transformation in organic synthesis to set up the carbon backbone of organic molecules. However, only a limited number of enzymatic C–C bond forming reactions have been applied in biocatalytic organic synthesis. Recently, further name reactions have been accomplished for the first time employing enzymes on a preparative scale, for instance the Stetter and Pictet–Spengler reaction or oxidative C–C bond formation. Furthermore, novel enzymatic C–C bond forming reactions have been identified like benzylation of aromatics, intermolecular Diels-Alder or reductive coupling of carbon monoxide.

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Introduction

Enzymatic carbon–carbon bond forming reactions [1] catalysed by aldolases, transketolases [2–5], hydroxynitrile lyases [1,6] and thiamin diphosphate (ThDP)-depending α-hydroxy ketone forming enzymes [1,7] are well established for synthetic purposes. This review focuses on C–C bond formation by enzymes, which are less established for biocatalysis, which have gained increased significance recently or which have been reported for the first time. The review covers the period of approximately the last two years. Subdivisions have been made according to the type of enzyme (lyase, oxidoreductase and transferase) and enzymes with promiscuous activity. Most examples belong to the group of lyases.

Lyases

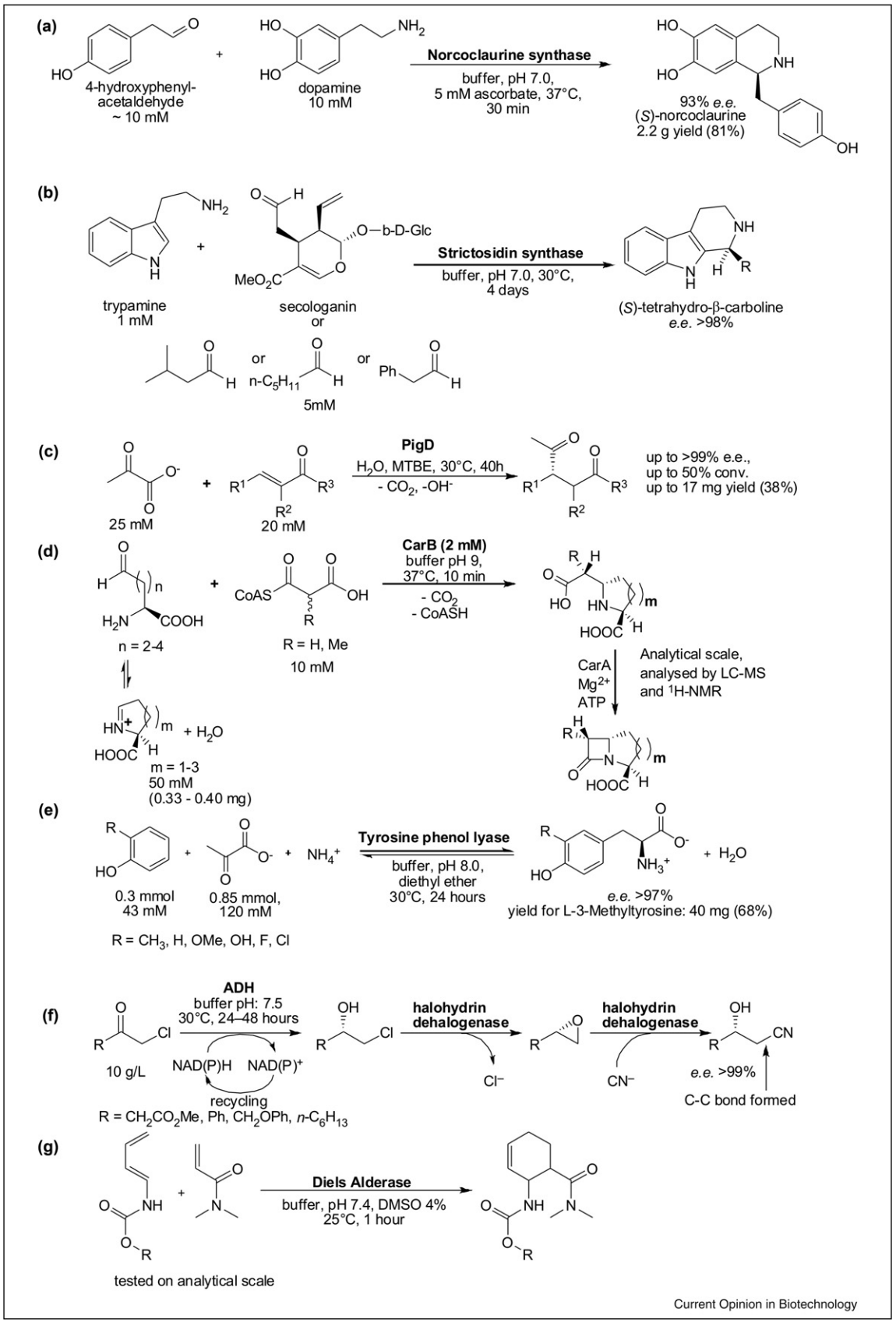
Pictet-Spenglerases. The group of 'Pictet-Spenglerases' [8] encompasses various enzymes such as norcoclaurine synthase and strictosidine synthase. The general reaction is the condensation of an aryl ethylamine with an aldehyde to form a six-membered *N*-heterocycle (Figure 1a,b). Norcoclaurine synthase (EC 4.2.1.78) catalyses the first step in benzylisoquinoline alkaloid biosynthesis by forming a C–C bond between dopamine and 4-hydroxyphenylacetaldehyde to yield (*s*)-norcoclaurine (Figure 1a). A recombinant norcoclaurine synthase originating from the plant *Thalictrum flavum* (meadow rue) [9,10] was used to prepare (*s*)-norcoclaurine starting from cheap tyrosine and dopamine as substrates in a one-pot, two-step process [11•]. Tyrosine was first chemically decarboxylated by stoichiometric amounts of sodium hypochlorite to generate the aldehyde species (4-hydroxyphenylacetadehyde), followed by the addition of the enzyme and dopamine substrate. The optimised process afforded (*s*)-norcoclaurine (*e.e.* 93%) in 81% yield and allowed the recycling of the enzyme.

Another Pictet-Spenglerase is strictosidine synthase (EC 4.3.3.2), which triggers in nature the formation of strictosidine from tryptamine and secologanin (Figure 1b). The recombinant enzyme from *Catharanthus roseus* was investigated concerning the acceptance of non-natural substrates [12] and a strictosidine synthase from *Ophiorrhiza pumila* was shown to accept a range of simple achiral aldehydes and substituted tryptamines to form highly enantioenriched (*e.e.* >98%) tetrahydro-β-carbolines [13•] (Figure 1b).

Thiamine diphosphate-dependent enzymes [7] were recently shown to catalyse besides the well-known formation of α-hydroxy ketones via 1,2-addition also a 1,4-addition when employing α,β-unsaturated ketones as substrates [14••] (Figure 1c). This remarkable new development allows exploiting the decarboxylation and umpolung of pyruvate to perform the so-called Stetter reaction giving access to 1,4-bifunctional molecules. This 1,4-addition was catalysed by the enzyme PigD from *Serratia marcescens*, which is in contrast to other ThDP enzymes, for example, the enzyme YerE, catalysing 1,2-addition. YerE on the other hand was successfully employed for the carboligation of ketones with pyruvate as reagent to form enantioselectively tertiary alcohols with a α-acetyl moiety [15•].

Crotonases. Enzymes of the crotonase superfamily catalyse a wide variety of reaction types including alkene hydration and isomerisation, coenzyme A ester hydrolysis and C–C bond cleavage. Two members of the superfamily have been reported to catalyse C–C bond formation [16], whereby in both cases the substrate bears a coenzyme A ester moiety. Carboxymethylproline synthase CarB from *Pectobacterium carotovorum* activates malonyl CoA derivatives via decarboxylation; the variant CarB His229Ala and its homologue ThnE from *Strepto-*

Figure 1



Biocatalytic C–C bond formation catalysed by lyases, such as **(a)** norcoclaurine synthase, **(b)** strictosidine synthase transforming non-natural substrates, **(c)** PigD, a ThDP-dependent enzyme, **(d)** CarB, a member of the crotonase superfamily, **(e)** tyrosine phenol lyase, **(f)** halohydrin dehalogenase, **(g)** computationally *de novo* designed Diels-Alderase.

myces cattleya [17] have been applied to convert amino acid aldehydes and malonyl CoA derivatives into 5-membered, 6-membered and 7-membered *N*-heterocycles (Figure 1d) [18••]. The products were converted further to bicyclic β-lactam derivatives by carbapenam synthetase CarA from *P. carotovorum*.

Tyrosine phenol lyase (EC 4.1.99.2), a pyridoxal 5-phosphate-dependent enzyme, catalyses *in vivo* the reversible β-elimination reaction of L-tyrosine leading to phenol, ammonium ion and pyruvate. Exploiting the reverse reaction, non-natural amino acids can be prepared from substituted phenols, pyruvate and ammonium (Figure 1e). Since the wild-type enzyme from *Citrobacter freundii* did not accept most investigated *o*-substituted phenols, variants were designed based on the crystal structure of the enzyme. The best-identified variant M379V allowed the synthesis of non-natural tyrosine derivatives possessing a chloro, methoxy or methyl substituent in position 3′ of tyrosine within one step [19•]. The obtained tyrosine derivatives are building blocks for bioactive compounds or biomarkers.

Halohydrin dehalogenases catalyse besides the ring-closure of vicinal halohydrins to the corresponding epoxides also the nucleophilic ring-opening of epoxides with a broad range of nucleophiles [20]. In case cyanide is used for the ring-opening of an epoxide, a new C–C bond is formed. Recently, a multi-enzymatic synthesis for the manufacture of atorvastatin (Lipitor®), a cholesterol-lowering drug, has been developed (Scheme 1f) [21,22]: After asymmetric reduction of ethyl 4-chloroacetoacetate by an alcohol dehydrogenase (ADH), the obtained halohydrin was converted into the epoxide and further into the corresponding hydroxynitrile by a halohydrin dehalogenase. Under optimised conditions, (*R*)-ethyl 4-cyano-3-hydroxybutyrate could be obtained in 95% isolated yield and *e.e.* > 99.9% with a space-time yield of 480 g L^{-1} day^{-1}. In a similar approach, a one-pot cascade was investigated, whereby by choosing the appropriate ADH, either enantiomer of various β-hydroxynitriles could be produced in good yield and optically pure form [23].

Diels-Alderase. So far no naturally occurring enzyme catalysing an intermolecular Diels-Alder reaction has been reported. Employing a computational approach (Rosetta computational design methodology) an enzyme was designed enabling the intermolecular Diels-Alder reaction of 4-carboxybenzyl *trans*-1,3-butadiene-1-carbamate and various acrylamide derivatives (Figure 1g) [24••]. The essential features of the active site to be established were hydrogen bond acceptor and donor groups to activate the diene and dienophile as well as complementary binding pockets to hold the two substrates in an optimal position. The designed genes (84) were synthesised with a His-tag and expressed in *Escherichia coli*. Fifty of the expressed enzymes were soluble and therefore chosen for purification. Out of these 50 enzymes, two enzymes were found to be active and mutations led to a 100-fold increase of the catalytic activity. The substrate spectrum of two variants was also tested using different dienophiles.

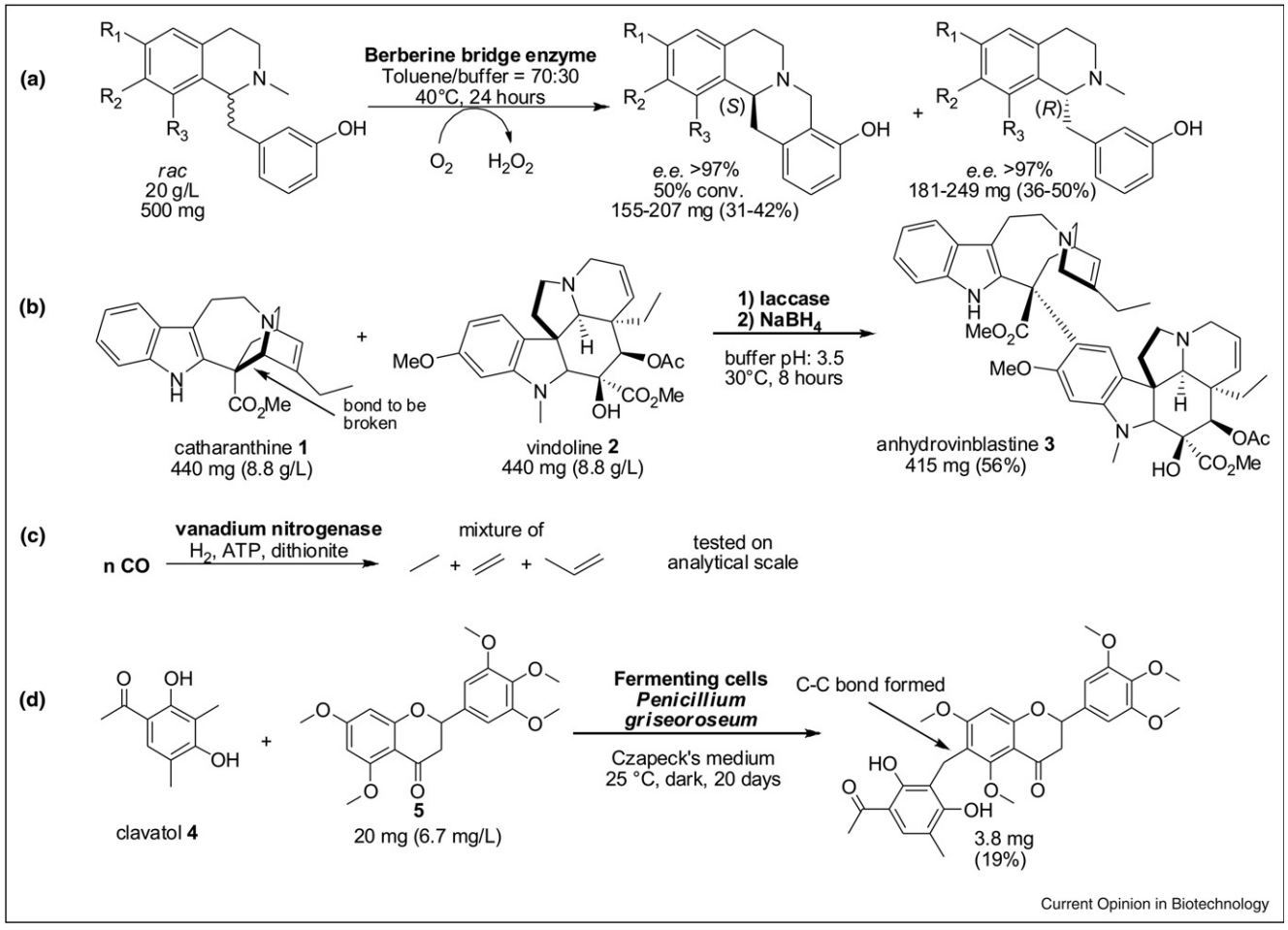
Oxidoreductases

Oxidoreductases are enzymes less commonly employed for C–C bond formation, except for laccases and peroxidases; however, none of these two enzymes controls the actual C–C bond formation reaction; they mediate just the formation of a reactive species. Nevertheless, novel redox enzymes like the *berberine bridge enzyme* (BBE; *vide infra*) were exploited for synthetic purposes and novel redox reactions for C–C bond formations were identified.

The BBE is involved in the biosynthesis of benzophenanthridine alkaloids in plants from the poppy family. It catalyses an intramolecular oxidative C–C bond formation between a phenol moiety and an *N*-methyl group at the expense of molecular oxygen. The most thoroughly studied enzyme is BBE from *Eschscholzia californica* (california poppy) which has been obtained in substantial amounts by overexpression in *Pichia pastoris*. Its X-ray crystal structure has been determined and the catalytic mechanism has been studied [25]. Recently, BBE has been employed for the preparation of novel optically pure (*R*)-benzylisoquinolines and (*S*)-berbine derivatives (Figure 2a) [26••]. Starting from a racemic mixture, exclusively the (*S*)-enantiomer was transformed via C–C bond formation leading to a kinetic resolution with perfect *E*-value ($E > 200$). The reaction could successfully be performed on a 500 mg scale at a substrate concentration of 20 g/L. Reactions were carried out in a toluene/buffer biphasic system to solubilise the substrates and O_2 was required as the stoichiometric oxidant.

Laccases are multi-copper containing enzymes that catalyse the oxidation of various *O*-substituted and *N*-substituted arenes at the expense of molecular oxygen [27]. They have been employed frequently for C–C bond formation to achieve polymerisation or oligomerisation, in ideal cases dimerisation [28]. Unfortunately, in many examples complex product mixtures were obtained [29]. The laccases oxidise, for example, the phenols to the corresponding highly reactive phenoxy radicals, which then can undergo various follow-up reactions, like C–C bond or quinone formation [30–32]. Thus, in general the laccase reaction on its own is not stereoselective. Nevertheless, an intriguing transformation mediated by a laccase was the synthesis of the complex bisindole alkaloid anhydrovinblastine **3** via the oxidative coupling of the indole derivatives catharanthine **1** and vindoline **2** and subsequent $NaBH_4$-reduction (Figure 2b) [33•]. The product could be isolated in 56% yield in optically pure

Figure 2



Oxidative C–C bond formation employing **(a)** the berberine bridge enzyme (BBE), **(b)** laccase, **(c)** vanadium nitrogenase, **(d)** fermenting cells for the benzylation of a flavanone derivative.

form. The remarkable reaction necessitates the initial cleavage of a C–C bond between a quaternary and a tertiary carbon centre in catharanthine **1**, followed by C–C bond formation to the quarternary carbon atom. Additionally, this is one rare example where the molecule to be oxidised has no phenolic alcohol moiety but a secondary amine. The $NaBH_4$ is supposed to be required for the reduction of an iminium group.

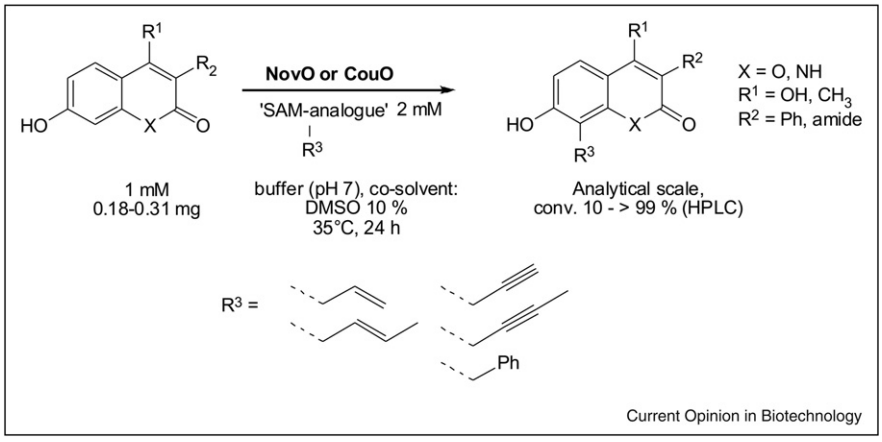
Vanadium nitrogenase. A recently reported, rather surprising example of C–C bond formation is the formation of ethylene, ethane and propane from carbon monoxide by vanadium nitrogenase in the presence of molecular hydrogen, ATP and dithionite as electron source [34•]. The holoenzyme originating from *Azotobacter vinelandii* comprises a catalytically active VFe-protein and a Fe-protein which serves as a reductant in the ATP-dependent electron-transfer process. At the moment this reaction is probably more a curiosity than a useful reaction to be applied on preparative scale.

Benzylation by fermenting cells. In the last redox example the enzymes involved are not characterised yet; only fermenting cells were employed. Nevertheless, from the overall reaction scheme it can be deduced that an oxidative step is definitely required and obviously C–C bond formation occurs. The fungus *Penicillium griseoroseum* was found to attach clavatol (**4**) to flavanone **5** at position C-6 (Scheme 2d) [35]. Thus, the overall reaction represents a benzylation of the flavanone. Flavonoids in plants are recognized as a part of their defence mechanisms, and therefore this observed C–C bond formation is suggested to represent an adaptation of fungi to plants' metabolite composition.

Transferases

Prenyltransferases will not be discussed here in detail, since they have recently been reviewed extensively [36,37] and have at the moment limited applications in biocatalytic organic synthesis. The same is true for methylation, which has been heavily investigated from a

Figure 3



Alkylation of coumarin derivatives employing methyltransferases with artificial SAM analogues.

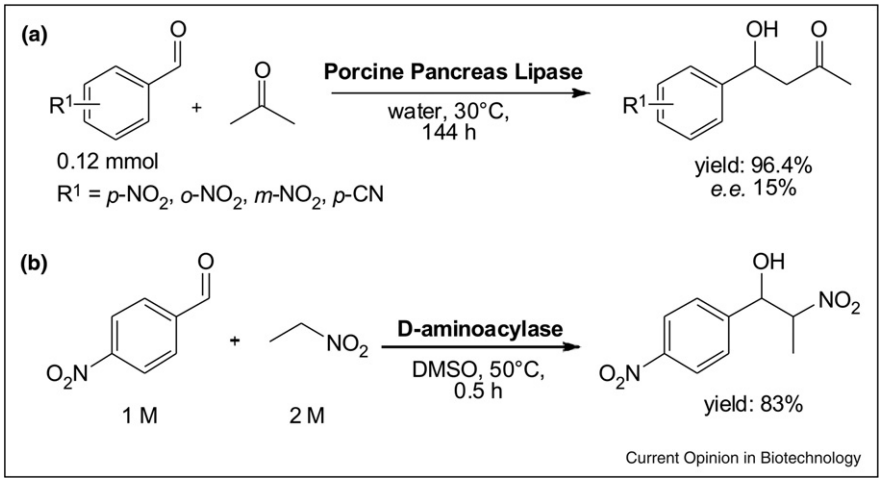
biochemical point of view [38,39]; however, synthetic applications employing methyltransferases are limited due to difficulties to recycle the required cofactor (SAM, S-adenoyl-L-methionine).

Nevertheless, a recent study indicated that methyltransferases have probably a significant synthetic potential: It has been shown that modified SAM-cofactors bearing an allyl, propargyl, but-2-en-1-yl, but-2-in-1-yl or benzyl group instead of methyl are also accepted by methyltransferases NovO from *Streptomyces spheroids* and CouO from *Streptomyces rishiriensis* (Figure 3) [40]. Thus, in addition to methylation at position **8** in coumarin derivatives, regioselective transfer of other alkyl groups (allyl, but-2-en-1-yl, but-2-in-1-yl, propargyl and benzyl) could be achieved.

Enzymes with promiscuous carbon–carbon bond forming activity

Catalytically promiscuous hydrolases were recently reviewed including promiscuous carbon–carbon bond formations [41]. In general, the C–C bond forming reactions catalysed by hydrolases do not yield products with a significant enantiomeric excess, actually the products are racemic in most cases. However, a lipase from porcine pancreas (Porcine pancreas lipase(PPL), EC 3.1.1.3) was reported recently to form the aldol product with an *e.e.* of 15% using 4-nitrobenzaldehyde and acetone as substrates (Figure 4a) [42]. The *e.e.* could be improved using less water in the reaction system so that an *e.e.* of 44% could be reached, but the conversion dropped significantly. In another example, nuclease p1 was used as a catalyst to perform the asymmetric aldol reaction

Figure 4



Carbon–carbon bond formation catalysed by enzymes with promiscuous activity: **(a)** lipase catalysing aldol reaction and **(b)** D-aminoacylase catalysing the Henry reaction.

between various aromatic aldehydes and cyclic ketones [43]. Depending on the substrates used, yields up to 55% with *e.e.* up to >99% and *d.r.* > 99:1 were achieved.

Probably initiated by a reported Henry reaction mediated by a hydroxynitrile lyase from *Hevea brasiliensis* [44,45], other enzymes have been shown to perform the biocatalytic Henry reaction as well: Examples are a transglutaminase (protein-glutamine L-glutamyltransferase; EC 2.3.2.13) from *Streptorerticillium griseoverticillatum* [46] and a D-aminoacylase (EC 3.5.1.81) (Scheme 4b) [47]. However, no results about the stereoselectivity were published.

Conclusion and perspective

The review demonstrates that the toolbox of enzymes catalysing various C–C bond forming reactions is expanding. It can be expected that many more C–C bond forming enzymes will be applied as biocatalysts in organic synthesis during the next years. Maybe soon non-natural products might be synthesised via elegant synthetic routes involving enzymatic C–C bond forming steps additionally to biocatalytic functional group modifications as is already common for biocatalytic synthesis of sugars.

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- •• of outstanding interest

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